THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

66TH STREET AND YORK AVENUE
NEW YORK 21, N.Y.

Jan 5, 1956

Dear Joshua,

I have sent you today a package containing:

1. Bresch agar dyes; Alizarol yellow GW conc.
National Fast Wool Blue R
These are vat dyes from National Aniline
Instructions:

To 300 cc of bottom agar (any complete medium) add just before pouring 7.5 cc of a glucose solution (60g glucose + 100 cc water); 1.5 cc of blue dye solution (2% w/v); 7.5 cc of yellow dye suspension (2.5% w/v). The yellow dye precipitates at room temperature. Shake to resuspend before adding and it will dissolve in the warm agar. No dye is added to the top layer. The dyes I assume are self sterilizing. The glucose can be autoclaved. The major difficulty with this medium is the depression of plaque size, with lambda this could be serious. However most of this is probably due to the excess acid from the glucose fermentation. It is my own feeling that the glucose concentration could be considerably reduced without affecting the unknown reactions.

2. Sw665 and Sr60 and a scoring reagent.

Sr60 is lysogenic derivative of 665 for a phage intentionally not quite "type" (y not +). It is stable and can be induced as well as TH2 lysogenic.

I am obviously retracting what I wrote in my last letter. The difficulty lay in certain peculiarities in the cross streak reaction for immunity and lysogenicity. At high multiplicities of phage the lysogenic is inhibited giving a pseudosensitive response. Still contaminated clones do not; thus the apparent segregation. The solution I have enclosed should easily differentiate sensitives and lysogenics. As the phage put out by the lysogenic does not grow well on parental 665 the interaction on cross-streaking is poor but scoreable. Can't use TH2 as 665 carries a phage attacking it as does the lysogenic . All scoring should be done on EMB 0 or EMB xylose. 665 and its derivatives grow slowly on this medium and will necessitate 48 hour or more incubation. This slow growth came in with the xylose mutation. You may recall that 541 was a mixture of xyl + and slow. The xyl- mutation was obviously in the xyl slow stock as it can be shown by transduction to differ from type by two genes. The phage mutational capacities are exhibited by all members of the 541 line. Have too much data with 665 to switch to 541.

Because of the critical nature of the experiment I shall also try my hand at delysogenizing Sr60 and will let you know immediately if I succeed. Hope one of us does.

Sincerely,

Alan